USING NITROGEN PROCESS INHIBITORS TO REDUCE AMMONIA VOLATILISATION AND NITROUS OXIDE EMISSIONS FROM LAND APPLIED DAIRY EFFLUENT

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Abstract

Dairy farm effluents (manure and liquid effluent) are commonly applied to soil as a source of organic nitrogen (N) fertiliser. However, these applications increase soil inorganic N, and may therefore increase ammonia (NH_3) volatilisation and nitrous oxide (N_2O) emissions. Various technologies have been used to reduce NH_3 and N_2O losses derived from agricultural N sources, including amending N sources with N process inhibitors such as urease inhibitors (UIs) and nitrification inhibitors (NIs). The objective of our study was to measure the effect of amending different forms of dairy effluent with UI - N-(n-butyl) thiophosphoric triamide (NBTPT, commercially named Agrotain® nitrogen stabiliser) and NI - dicyandiamide (DCD). Two field experiments were conducted to study the effect of these inhibitors on NH_3 and N_2O emissions. Treatments included fresh manure, stored manure, fresh farm dairy effluent (FDE) and stored FDE, with and without Agrotain® (0.25 g/kg N) or DCD (10 kg/ha), applied in late-winter on a free-draining volcanic soil in the Waikato region. The application rate of the FDE and manure, which had different dry matter contents (2% and 22.5%, respectively) was about 100 kg N ha^{-1}. Results showed that application of manure and FDE, both in fresh and stored forms, to pasture led to NH_3 volatilisation (15, 1, 17 and 0.4% of applied N in fresh manure, fresh FDE, stored manure and stored FDE lost as NH_3, respectively). Adding UI had a significant effect ($P < 0.05$) on reduction of NH_3 losses from both fresh manure and fresh FDE, decreasing NH_3 emissions by 48% and 38%, respectively. All types of effluent have the potential to produce N_2O, particularly fresh manure and fresh FDE. The emission factors (amount of N_2O-N emitted as a percentage of applied N) of fresh manure, fresh FDE and stored FDE were 0.13%, 0.14% and 0.03%, respectively. DCD was effective in decreasing N_2O emissions in the stored FDE, fresh FDE and fresh manure by 90%, 51% and 46%, respectively. This study showed that NBTPT can be effective in mitigating NH_3 losses and DCD in reducing N_2O emissions from land applied dairy effluents.

Key words: Ammonia, Nitrous oxide, Farm dairy effluent, Manure, Pasture, Nitrogen

Introduction

One of the challenges facing the dairy industry due to its rapid intensification is the need for proven effluent management systems that increase nutrient use efficiency and minimize off-farm environmental impacts. Increased animal numbers, greater use of fertiliser and higher supplementary feed inputs on dairy farms have markedly changed the volume and types of effluent produced. Effluent contains significant quantities of valuable nutrients that, if applied to land, could improve soil fertility and increase the resource use efficiency of farming systems (Luo *et al.*, 2008; de Klein *et al.*, 2011). A better understanding of effluent nutrient

dynamics from different products with a range of storage and application options will allow the dairy industry to design effluent management systems to increase nutrient use efficiency. Environmental concerns from applying effluent encompass all aspects of non-point source pollution, including contamination of surface waterways and groundwater, reduced air quality by emission of volatile organic compounds, and the increased emission of ammonia (NH_3) and nitrous oxide (N_2O) (Bolan *et al.*, 2004). Application of inorganic N fertilizer may lead to NH_3 and N_2O emissions (Zaman and Blennerhassett, 2010a; Halvorson *et al.*, 2011; Massey *et al.*, 2011), but the relative emissions from different types of dairy effluent is poorly understood because only a limited number of comparative studies have been conducted (Williamson *et al.*, 1996; Chadwick *et al.*, 2000; Luo *et al.*, 2008). Various approaches could be used to reduce NH_3 and N_2O losses from manure and FDE. One approach is the use of urease inhibitors (UIs) and nitrification inhibitors (NIs). N-(n-butyl) thiophosphoric triamide (NBTPT, commercially named Agrotain®) is a urease inhibitor which can form a tridentate ligand to slow urea hydrolysis, reducing NH_3 volatilisation (Watson *et al.*, 1990). The nitrification inhibitor dicyandiamide (DCD) retards the rate at which ammonium (NH_4^+) is converted to nitrate (NO_3^-), which can lead to a reduction in N_2O emissions. UI and NI have been shown to reduce N losses from animal urine on grazed pasture and applied N fertilizer (Di and Cameron, 2012; Sanz-Cobena *et al.*, 2012; Zaman and Nguyen, 2012). However, it is not clear if NBTPT and DCD can be used to reduce NH_3 and N_2O losses from applications of dairy effluent on to pasture.

The objectives of this study were 1) to quantify gaseous NH_3 and N_2O losses from different forms of dairy effluent application to pasture soil in late winter, and 2) to evaluate the potential of NBTPT or DCD to reduce gaseous emissions of NH_3 or N_2O, respectively, from applications of different forms of effluent.

Materials and methods
Experimental site
Two field plot trials were conducted at AgResearch's Ruakura No.1 dairy farm in Hamilton, New Zealand, between August, 2012 and January, 2013. The first was to measure NH_3 emissions, while the second focused on N_2O emissions. The site has temperate a temperate climate, with about 1240 mm annual rainfall and mean annual temperature of $14°C$. The field site supports sheep and beef-grazed (10-12 s.u. ha^{-1} yr^{-1}) rotationally grazing ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture on a Typic Impeded Allophanic Soil called Bruntwood silt loam (Hewitt, 1992). Animals were excluded from the trial site for at least six weeks prior to application of treatments to avoid effects from urine or dung N deposition. Table 1 provides details of the soil properties at the experiment site.

Table 1 *Chemical and physical characteristics of soil from the field trial (10 cm depth)*

	Total N (%)	Bulk density ($Mg\ m^{-3}$)	C.E.C ($cmol_c\ kg^{-1}$)	Organic C (%)	Organic matter (%)	pH
Soil	0.95	0.72	11.2	9.3	16	6.0

Effluent
Four types of effluent were used in this study; stored manure, fresh manure, stored FDE and fresh FDE. Stored manure and FDE that had undergone four months storage were collected from local commercial dairy farms in the Waikato region. Fresh manure was made by mixing fresh dairy cow urine and dung at the ratio of 1:1.3 (urine: dung), based on an average cow

excreting 7000 L urine and 9000 kg dung wet weight year^{-1} (Haynes and Williams, 1993). Fresh FDE used in the trial was made by mixing fresh dairy cow urine and dung at the ratio of 1:1.3 (urine: dung), and diluting with water to give an effluent of about 2% dry matter (DM). Urine was collected from dairy cows at the AgResearch Ruakura No. 1 dairy farm during milking, while dung was collected from a freshly grazed paddock at the Dairy New Zealand's Scott farm. Dung and urine were separately bulked, mixed and applied on the plots. Subsamples of each effluent type were taken for chemical analysis (Table 2).

Table 2 *Characteristics of stored manure, stored FDE, fresh FDE and fresh manure used in the trials*

	TKN (%)	NH$_4^+$-N (mg kg^{-1} soil)	K (mg kg^{-1} soil)	Organic C (%)	Dry matter (%)	pH
Experiment 1 (NH$_3$)						
Fresh FDE	0.11	450	890	0.5	1.6	8.7
Stored FDE	0.11	120	300	1.0	3.1	7.2
Fresh manure	0.52	920	4740	2.8	9.2	8.0
Stored manure	0.47	1940	5500	3.8	12.3	7.8
Experiment 2 (N$_2$O)						
Fresh FDE	0.12	460	970	0.6	1.8	8.8
Stored FDE	0.09	110	390	0.6	2.1	7.0
Fresh manure	0.55	2160	4650	3.0	9.5	8.0

Experimental details

The first experiment was designed to compare emissions of NH$_3$ from fresh FDE (with or without urease inhibitor), fresh manure (with or without urease inhibitor), stored FDE and stored manure. The second experiment was to compare emissions of N$_2$O from fresh FDE (with or without DCD), fresh manure (with or without DCD) and stored FDE (with or without DCD). A non-effluent treatment as a control (C) was also included. In both experiments, there were four replicates of each treatment. All treatments were applied in a randomised block design.

The application rate of FDE or manure in all treatments was equivalent to about 100 kg N ha^{-1}. The UI (0.25 g kg^{-1} N) and NI (10 kg ha^{-1}) were mixed with the different forms of effluent and evenly distributed over the entire sampling chamber (15 cm diameter for the NH$_3$ chamber and 24 cm diameter for the N$_2$O chamber). NH$_3$ emissions were measured for 27 days after the treatments were applied in August 2012, and N$_2$O emissions analysed for 172 days.

Ammonia volatilisation was measured using an enclosure method similar to that used by Sherlock and Goh (Sherlock and Goh, 1985). Once the treatments were applied, enclosures consisting of a 15 cm diameter PVC pipe with a clear perspex lid and sharpened base were pushed 2 cm into the ground, leaving a headspace in the enclosure of 3 cm. A pump drew air through the enclosure before passing it through a dreschel bottle containing 250 ml of 0.05 M sulphuric acid to trap any NH$_3$ present. The enclosures were left in place with the pump

running continuously for 27 days. The acid solution was sampled and replaced every 24 hours for the first five days and thereafter at 2-7 day intervals through to 27 days. The NH_3 flux (mg N m^{-2} hr^{-1}) was calculated using the following equation:

$$NH_3 \text{ flux} = C \times V / (D \times A) \tag{1}$$

Where, NH_3 flux= mg N hour^{-1}; C = NH_3 concentration in the acid trap (mg dm^{-3}); V = is the volume of the acid (dm^3); D = duration (hours) of each sampling; A= area of enclosure (m^2)

A soil chamber technique was used to measure N_2O emissions, and the methodology was based on that from the study of Luo et al. (2013). The chambers were modified PVC 'sewer-hatches' (24 cm diameter and 30 cm high) with an internal half-turn locking system and a greased rubber O-ring. Chambers were inserted 5-10 cm into the soil before the treatment application. Gas sampling occurred twice per week for the first four weeks and was then reduced to once per week for approximately 21 weeks. On each sampling day, the chamber was closed with a lid for 1 h (between 11am to 1pm), and the air above the soil surface was sampled through a three-way tap on the chamber lid, using a 50-ml syringe. A 12-ml air sample was taken from each chamber at 0 minutes (T_0) and 60 minutes (T_{60}) for N_2O analysis.

The gas samples were analysed for N_2O concentration using a Shimadzu GC-17A gas chromatograph equipped with a ^{63}Ni-electron capture detector (oven, valve and detector temperatures operated at 65, 100 and 280°C, respectively) with oxygen-free nitrogen as a carrier gas.
The hourly N_2O fluxes (mg N m^{-2} h^{-1}) were calculated from the increase in head space N_2O over the sampling time:

$$N_2O \text{ flux} = \frac{\delta N_2O}{\delta T} * \frac{M}{Vm} * \frac{V}{A} \tag{2}$$

Where δN_2O is the increase in head space N_2O over time (μL/L); δT is the enclosure period (hours); M is the molar weight of N in N_2O; Vm is the molar volume of gas at the sampling temperature (L/mol); V is the headspace volume (m^3); and A is the area covered (m^2).

Emission factors were then calculated from the difference in total emissions from each effluent treatment and the control treatment, divided by the rate of effluent N applied, as described by the following equation:

$$EF (\%) = [N_2O \text{ total (effluent)} - N_2O \text{ total (control)}] / FDE \text{ N applied} \times 100 \tag{3}$$

Where EF is emission factor (N_2O-N emitted as a % of effluent-N applied), N_2O total (effluent) and N_2O total (control) are the cumulative N_2O emissions from the effluent and control plots, respectively (kg N ha^{-1}). Effluent N applied is the rate of different types of effluent N applied (kg N ha^{-1}).

Statistical analyses
An analysis of variance was performed using SPSS software package for Windows (Version 13.0, SPSS inc, Chicago, IL, USA), and least significant differences (LSD) were calculated. Significant differences between two treatments were defined at $P < 0.05$.

**Results and discussion**

Ammonia emissions

Ammonia emissions from the control treatment were negligible throughout the experiment. The addition of different types of effluents led to NH_3 emissions within one or two days of application (Figure 1). This observation was consistent with other studies (Sharpe and Harper, 1997; Zaman and Nguyen, 2012) where most of the applied N is converted to ammonium within the first two days after effluent application. The stored manure treatment increased the NH_3 emission within 24 hours of application, reaching a maximum value of 13.1 kg N ha^{-1} day^{-1}. During the first two days of the trial, ammonia volatilisation from fresh FDE, stored FDE and fresh manure was significantly ($P < 0.05$) less than that from stored manure. The amount of NH_3 emitted and the peak emission were higher in manure than in FDE treatment. The difference in the NH_3 emission pattern between these N sources (manure and FDE) was probably due to the differences in the content of dry matter in effluents. The content of dry matter in effluents has been shown to be an important factor in determining NH_3 volatilisation potential (Sommer and Hutchings, 1995; Smith *et al.*, 2000), with effluents higher in dry matter content showing greater ammonia loss (Sommer and Olesen, 1991). This relationship is due to the fact that the manure with higher dry matter was on the soil surface longer, giving more chances for the release of NH_4^+ ions as NH_3. However, FDE with lower dry matter tend to have greater fluidity and, therefore, infiltrate more readily into the soil where NH_4^+ is protected from volatilization by adsorption onto soil colloids (Sharpley *et al.*, 1998). Effluent characteristics like ammonium-N concentration can also affect ammonia emissions (Rotz, 2004). In this experiment, fresh FDE and manure which have urea were rapidly hydrolysed and can lose NH_3. However, stored manure which had the highest NH_3 emissions would have had little urea left, and losses may be more due to high NH_4^+-N concentrations in stored manure (0.194 %) (Table 2). Similarly, in a laboratory study of 18 poultry litters in Delaware, Schilke-Gartley and Sims (1993) found potential ammonia volatilization to vary from 4 to 31% of manure total N, with strong correlations between ammonia loss and NH_4^+-N concentrations ($R^2 = 0.77$). In all the treatments, NH_3 emissions dropped quickly during the first two days and then gradually declined to background levels.

Addition of UI resulted in a significant reduction ($P < 0.05$) and delay in NH_3 emissions from fresh FDE and fresh manure treatments (Figure 1). During the first five days of the trial, ammonia volatilisation from fresh manure treated with NBTPT was significantly lower ($P < 0.05$). The factors contributing to the effectiveness of NBTPT in controlling NH_3 emissions through the inhibition of urea hydrolysis include (i) increased time for the urea to diffuse deeper into the soil, and (ii) increased opportunity for rain or irrigation to dilute urea and NH_4^+ concentrations at the soil surface and increase its dispersion in the soil (Watson *et al.*, 1998). NBTPT was less effective in reducing NH_3 loss from fresh FDE than from fresh manure; this could be attributed to the fresh FDE having higher water contents, which in turn transported the effluent nitrogen to greater depths in the soil, leading to a relatively low N level on the soil surface, giving less chance for the release of NH_4^+ ions as NH_3. However, from day 5 to 15 the fresh manure with NBTPT addition had higher volatilisation losses than the pure fresh manure treatment ($P > 0.05$). After that, both treatments declined to background levels. The emissions from fresh FDE were reduced by NBTPT from day 2 to 20 ($P < 0.05$), after which they all decreased to background levels.
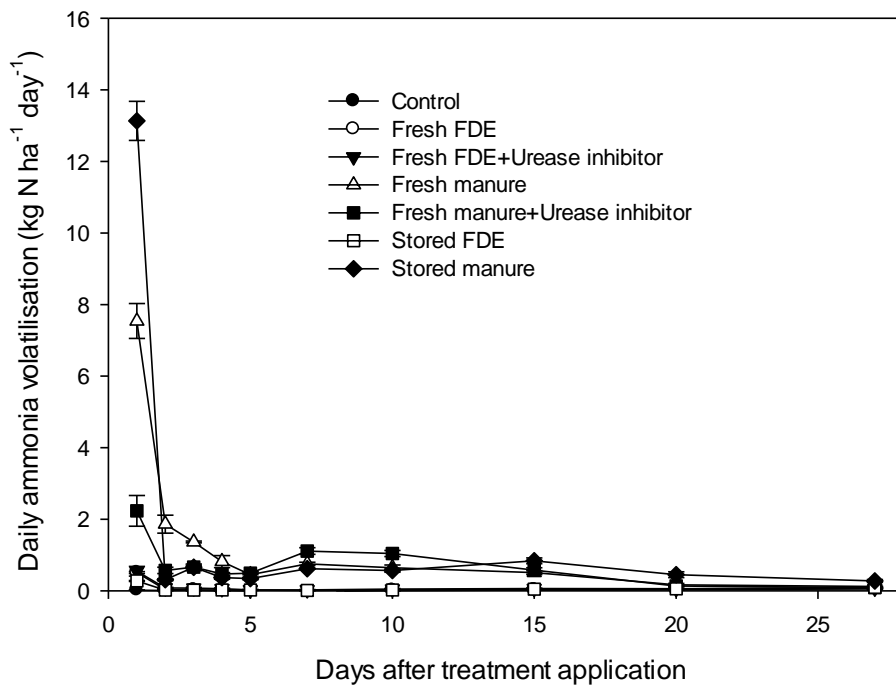
Figure 1 *Daily ammonia emission fluxes from different types of effluent applications, with and without urease inhibitor. Error bars represent standard error of the mean.*

Over the full 27 day period, the stored manure treatment lost 18% of the applied N as NH_3, which was significantly higher ($P < 0.05$) than the other treatments (Figure 2). The fresh manure treatment lost 15% of applied N, while 8% was lost when the urease inhibitor NBTPT was added, resulting in a 48% reduction in NH_3 emissions ($P < 0.05$). The application of NBTPT to fresh FDE resulted in a slightly lower proportion of N volatilised (0.7 kg ha^{-1}), compared to the fresh FDE only treatment (1 kg ha^{-1}) (Figure 2).
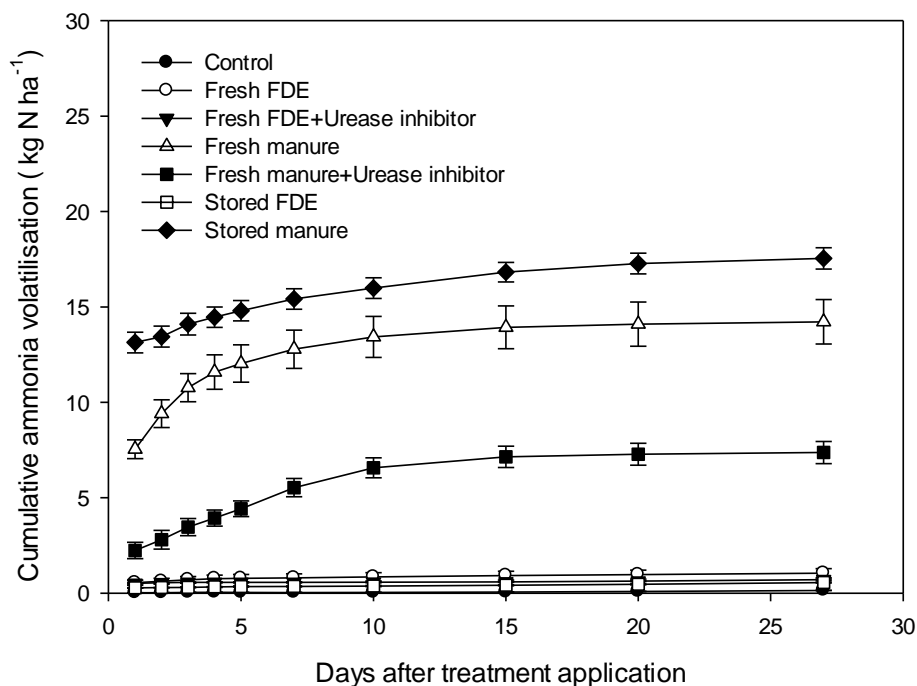
Figure 2 *Cumulative amount of applied N volatilised as ammonia from different types of effluent, with and without urease inhibitor. Error bars represent standard error of the mean.*

Nitrous oxide emissions

The rate of N_2O emission from the control treatment was very low, remaining between 0.001 and 0.007 mg N_2O-N m^{-2} hr^{-1} (Figure 3). Fresh FDE, fresh manure and stored FDE applications resulted in peak N_2O emissions within the first 4 days, followed by a progressive decline with time. The increase in N_2O emissions following different types of effluent application observed in our study is consistent with other studies that have measured N_2O emissions from soils amended with animal slurries (Velthof and Oenema, 1993; Chadwick *et al.*, 2000). With fresh FDE, the N_2O emission was higher than from other treatments until 9 days after application. In the fresh manure treatment, the highest emission of 0.066 mg N_2O-N m^{-2} hr^{-1} was observed on day 4 but then decreased markedly (Figure 3). Addition of stored FDE resulted in a small initial peak (0.011 mg N_2O-N m^{-2} hr^{-1}) on day 4, and emissions then remained lower than other treatments. Overall, cumulative N_2O emissions were in the following order: fresh FDE > fresh manure > stored FDE ($P < 0.05$). Some researchers have attributed increasing N_2O emissions to increasing C content in FDE (Jagrati *et al.*, 2008). The greater C contents (Table 2) in fresh manure (2.8%) and fresh FDE (1.0%) may have enhanced denitrification to a greater extent than for stored FDE (0.5%).
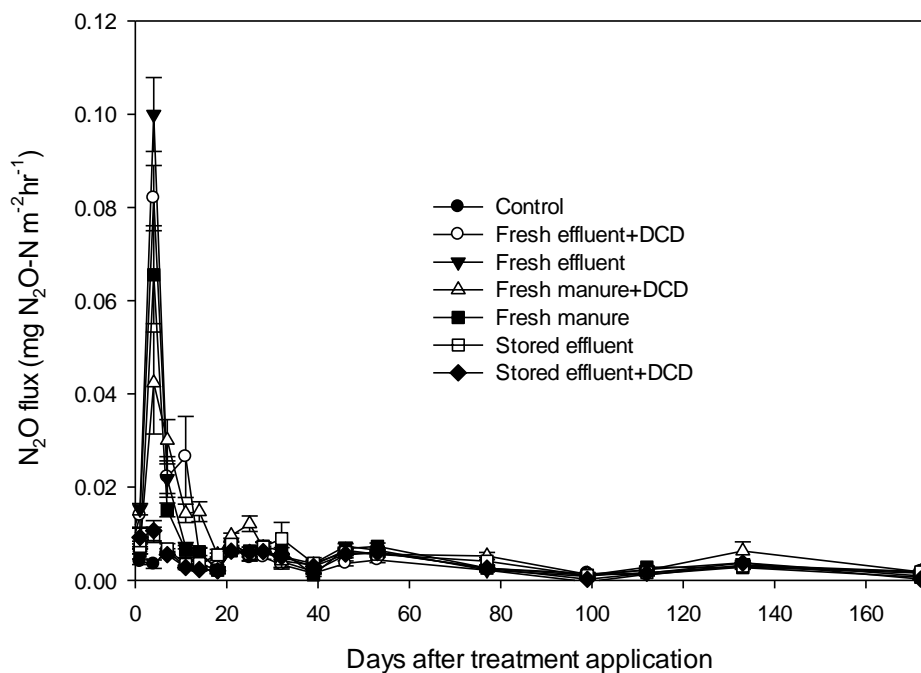
Figure 3 *N_2O emissions as affected by different types of effluent, with and without DCD. Error bars represent standard error of the mean*

The addition of DCD reduced and delayed N_2O emissions from all forms of applied effluent. In the fresh FDE treatment, the peak N_2O emission flux was reduced from 0.1 to 0.08 mg N_2O-N m^{-2} hr^{-1} with added DCD ($P < 0.05$). However, from day 8 to 16, the fresh FDE with DCD treatment had higher N_2O losses than the fresh FDE treatment alone. Addition of DCD to fresh manure lowered peak N_2O emission on day 4 ($P < 0.05$), but subsequent N_2O emissions were higher than in the fresh manure-only treatment. The application of DCD to stored FDE reduced cumulative N_2O emissions during the entire experimental period ($P < 0.05$). Others (Merino *et al.*, 2002; Di and Cameron, 2006) also found that DCD reduced N_2O

emissions when added to cattle urine and cattle slurry. Hatch et al. (2005) reported that mixing of DCD with cattle slurry reduced N_2O emissions by 96% compared to slurry only. In contrast, Williamson and Jarvis (1997) found no difference in N_2O emissions when DCD was added to dung and stored wastewater. The contrasting results are not unexpected since the effectiveness of DCD is known to be highly dependent on soil and environmental factors such as temperature. DCD is more effective at temperatures < 20°C (Prasad and Power, 1995). The temperature in this study was between 8 and 16 °C, which might suggest a slow DCD degradation rate (Figure 4). However, after day 8, DCD had no significant effect on N_2O emissions. Rainfall occurred on days 8 to 12, which may have leached DCD out of the surface soil where denitrification and N_2O production occurred (Figure 4). DCD separation from NH_4^+ sites in the surface soil and subsequent leaching under excessively wet soil conditions are likely to reduce DCD effectiveness (Zaman and Blennerhassett, 2010b; Kim *et al.*, 2012). In addition, the high organic matter content of the soil (9.3%) (Table 1) may have reduced the inhibitory effect of DCD. When soil organic matter is ≥ 5%, the inhibitory effect of NIs reduces due to the effect of organic matter on increased sorption and the rate of decomposition of NIs (Prasad and Power, 1995).
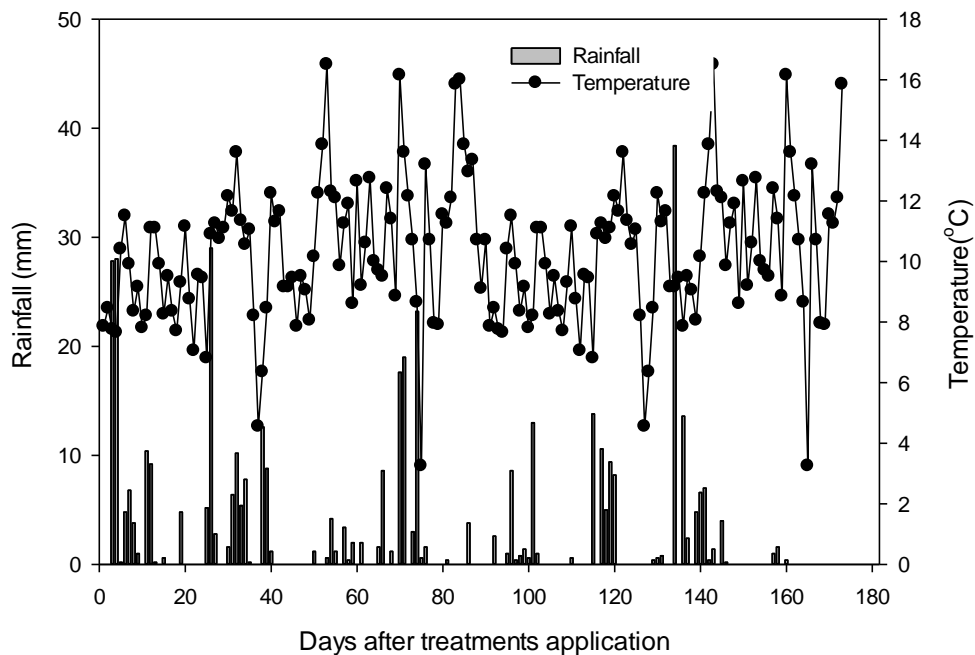

Figure 4 Daily rainfall and soil temperature at 5 cm depth during the experiment

The emission factor (EF) calculated for stored FDE (0.03%) was less ($P < 0.05$) than that for fresh manure (0.13%) and fresh FDE (0.14%) (Table 3). DCD application to the soil significantly ($P < 0.05$) reduced the EF for fresh FDE, fresh manure and stored FDE by a 51%, 46% and 90%, respectively (Table 3).

Table 3 N_2O emissions from application of dairy effluents. (The N application rate was 100 kg N ha^{-1}). Treatments with different letters in a column are significantly different ($P < 0.05$)

Treatment	N_2O emissions (kg N_2O-N ha^{-1})	Emission factor (%)	Reduction by DCD (%)
Control	0.13d	-	
Fresh FDE	0.26a	0.14	-
Fresh FDE+DCD	0.20b	0.07	51%
Fresh manure	0.25a	0.13	-
Fresh manure + DCD	0.20b	0.07	46%
Stored FDE	0.16c	0.03	-
Stored FDE+DCD	0.13d	0.00	90%

Conclusions

This study showed that applying different types of effluent to pasture soil led to varying NH_3 emissions, with greater emissions being found from stored manure and fresh manure compared to stored FDE and fresh FDE at the same loading rate of N. This was attributed to differences in the content of dry matter and ammonium in the applied effluents. The urease inhibitor NBTPT decreased NH_3 losses from both fresh manure and fresh FDE. All types of effluent have the potential to produce N_2O, particularly fresh manure and fresh FDE. Addition of DCD decreased N_2O emissions from land-applied fresh FDE, stored manure and fresh manure treatments. This study illustrates the potential benefits from NBTPT in mitigating NH_3 losses, and from DCD in mitigating N_2O emissions from dairy effluents.

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